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Conservation of Molecular Structure of DNA Polymerase β in Vertebrates Probed by Tryptic Peptide Mapping[1]

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DNA polymerase β's from mouse myeloma, chick embryo, and cherry salmon testis were all composed of a single polypeptide of about 40K daltons as judged by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis of extensively purified enzyme preparations. Although the enzyme from bullfrog ovary was not fully purified, its molecular weight was estimated to be the same as that of the chick enzyme by immunological detection after electrophoresis. All the enzymes tested cross-reacted immunologically with the antibody against chick DNA polymerase β, indicating that they have a common molecular structure, at least in part.

Two-dimensional maps of radioiodinated tryptic peptides directly showed the presence of highly conserved amino acid sequences among mouse, chick, and cherry salmon enzymes. This conserved structure is thought to be essential for the enzyme activity, which is very similar among all these vertebrates.

DNA polymerase β [EC 2.7.7.7] has been purified from many kinds of animal sources. The enzymatic properties of these preparations are very similar to each other even though the animal species or classes are different. Chang *et al.* (*1*, *2*) showed the evolutionary conservation of the structure of this enzyme by using an immunological detection method. We have demonstrated the existence of extensive structural homology among the enzyme preparations from various mammalian and avian tissues including calf thymus, swine liver, rabbit liver, rat liver, rat ascites hepatoma, mouse myeloma, and chick embryo by the two-dimensional tryptic peptide mapping method (*3*, *4*).

Our previous paper (*5*) showed that DNA polymerase β purified to homogeneity from cherry salmon testis has about the same molecular size as and similar enzymatic properties to the enzymes from other animal sources. In this report, we demonstrate the presence of very high structural

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homology of the enzyme among all the vertebrates by using immuno-blotting and tryptic peptide mapping methods. In the peptide maps, most of the spots that are common between the salmon and chick enzymes are also common between the chick and mouse enzymes. These results suggest that the primary structure of the essential core responsible for DNA polymerase activity is strongly conserved among all vertebrates, from fish to mammals.

MATERIALS AND METHODS

Cells and Tissues—All mammalian cells and tissues (*3*), chick embryo (*4*), and cherry salmon (*Oncorhynchus masou*) testis (*5*) were obtained as described previously. Ovaries of bullfrog (*Rana catesbeiana*) were taken from young females and kept frozen at −80°C until use.

Chemicals—Unless otherwise specified, chemicals used in the present study were obtained or prepared as described previously (*3*, *4*). Na[^{125}I]-iodide was purchased from New England Nuclear, Boston, Mass. Nitrocellulose membrane was from Schleicher & Schuell, W. Germany.

Enzymes—Trypsin and pancreatic DNase were from Boehringer Mannheim, W. Germany. DNA polymerase β from mouse myeloma MOPC104E (*3*), rat ascites hepatoma (*6*), chick embryo (*4*), and cherry salmon testis (*5*) were purified to near homogeneity as described previously.

Antibody—Anti-chick DNA polymerase β antibody from a rabbit was obtained as described previously (*7*). Horseradish peroxidase-conjugated goat anti-rabbit IgG was from Cappel Laboratories, Cockranville, Pa.

Assay of DNA Polymerase β—Assay no. 1: The reaction mixture (25 μl) contained the following: 50 mM Tris-HCl, pH 8.7 at 37°C, 10 mM Mg-acetate, 1 mM dithiothreitol, 100 μM each of dATP, dGTP, and dCTP, 50 μM [^{3}H]dTTP (120 cpm/pmol), 15% (v/v) glycerol, 400 μg/ml bovine serum albumin, 80 μg/ml activated calf thymus DNA, 50 mM KCl, and 1 or 2 μl of enzyme fraction.

Assay no. 2: The reaction mixture (25 μl) contained the following: 50 mM Tris-HCl, pH 8.5 at 37°C, 0.5 mM $MnCl_2$, 1 mM dithiothreitol, 100 μM [^{3}H]dTTP (60 cpm/pmol), 40 μg/ml each of $(rA)_n$ and $(dT)_{12-18}$, 12% glycerol, and 1 or 2 μl of enzyme fraction.

After incubation for 15 or 30 min at 37°C, the reaction mixture was transferred to a DEAE-cellulose paper disc (Whatman DE81), and the disc was washed as described by Lindell *et al.* (*8*). One unit of the enzyme activity was defined as the amount catalyzing the incorporation of 1 nmol of [^{3}H]dTMP into polymer DNA in 60 min.

Neutralization of the Enzyme Activity by Anti-Chick DNA Polymerase β Antibody—For the neutralization of DNA polymerase β activities from chick, mouse, and salmon by anti-chick β antibody, the enzyme, and the indicated amount of immunoglobulin were mixed and preincubated for 4 h at 0°C, then the reaction mixture for assay no. 2 was added, and the whole was incubated for 15 min at 37°C.

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis—Polyacrylamide gel electrophoresis was carried out essentially as described by Laemmli (*9*) using a slab gel of 10% (constant) or 7.5 to 15% (linear gradient) acrylamide concentration.

Electrophoretic Transfer of Proteins from a Gel to a Nitrocellulose Membrane—Blotting of proteins from a gel to a nitrocellulose membrane was carried out essentially by the method of Towbin *et al.* (*10*). In brief, the slab gel after electrophoresis was soaked in blotting buffer (25 mM Tris, 192 mM glycine, pH 8.3, 20% methanol) and a sheet of presoaked nitrocellulose membrane was layered on the gel, then a sandwich was made up. Electrophoretic transfer was carried out at 10 V/cm for 4 h with cooling.

Immuno-Peroxidase Staining—After blotting, the nitrocellulose membrane was treated with 2% bovine serum albumin (BSA) in TBS (Tris-buffered saline containing 50 mM Tris-HCl, pH 8.0 and 0.9% NaCl) for 1 h at room temperature and subsequently with 10% swine serum in TBS to block the protein binding capacity of the membrane. The membrane was then treated with 10 μg/ml of rabbit anti-chick DNA polymerase β IgG in 0.1% BSA-TBS for 2 h at room temperature. The membrane was washed six times for 3 min each with ice-cold TBS to remove non-binding antibody. The membrane was then treated with 5 μg/ml of horseradish peroxidase-conjugated goat anti-rabbit IgG in 0.1% BSA-TBS for 1 h at room temperature and washed six times with ice-cold

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TBS. The polypeptides that reacted with anti-chick DNA polymerase β antibody were visualized by staining the membrane with 0.5 mg/ml 3,3'-diaminobenzidine-HCl and 0.05% H_2O_2 made up in TBS.

Tryptic Peptide Mapping—Radioiodination, digestion with trypsin and two-dimensional separation of peptides on thin layer plates were carried out as described by Elder *et al.* (*11*) with some modifications (*3*, *4*).

RESULTS

Comparison of the Sizes of DNA Polymerase β from Various Vertebrates—The apparent molecular weights of the polypeptides of DNA polymerase β's from various animal tissues were estimated and compared by SDS-polyacrylamide gel electrophoresis with dye staining or with immunoperoxidase staining after electrophoretic transfer of the polypeptides to a nitrocellulose membrane. Panel A of Fig. 1 shows the Coomassie blue stain-

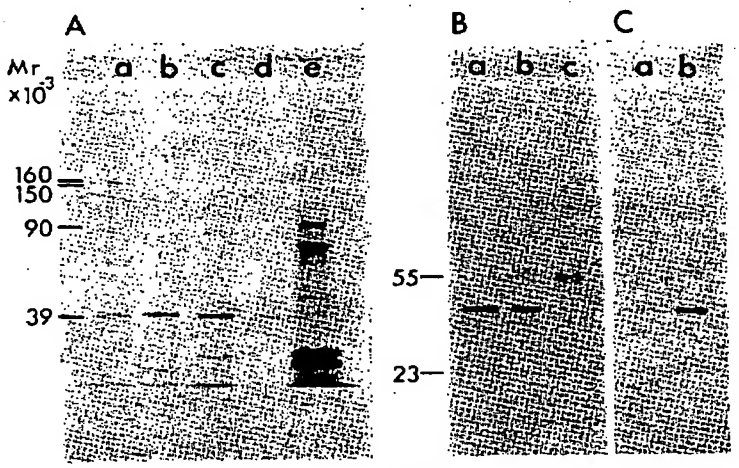


Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein-blotting of DNA polymerase β from various animal sources. Panel A: SDS-polyacrylamide gel electrophoresis. Extensively or partially purified enzymes from mouse myeloma MOPC104E (Lane b), chick embryo (Lane c), cherry salmon testis (Lane d), and bullfrog ovary (Lane e) were run on a single 10% slab gel at 10 mA constant current for 3 h. Note that the sample from bullfrog ovary was not pure enough for the enzyme band of 40K daltons to be detectable (Lane e). Lane a is RNA polymerase from *Escherichia coli* as molecular weight markers: β' (160K daltons), β (150K daltons), γ (90K daltons), and α (39K daltons) subunits. Panel B: Protein-blotting and immuno-peroxidase staining of DNA polymerase β. Partially purified preparations of polymerase β's from bullfrog ovary (Lane a; the same preparation as shown in Panel A, 70 units) and chick embryo (Lane b; less pure preparation than that shown in Panel A, 40 units) were separated on SDS-polyacrylamide gel (10%), then transferred onto a nitrocellulose membrane electrophoretically. The membrane was treated with anti-chick DNA polymerase β antibody, and then stained by the immuno-peroxidase method described in "MATERIALS AND METHODS." Lane c is rabbit IgG as molecular weight markers: H chain (55K daltons) and L chain (23K daltons). Panel C: Blotting and immuno-peroxidase staining of salmon DNA polymerase β. Extensively purified polymerase β from cherry salmon testis (Lane a, 200 units) and chick embryo (Lane b, 60 units) were run on SDS-polyacrylamide gel, and transferred to a nitrocellulose membrane. The membrane was treated and stained as described above.

ing of the electrophoresis gel of DNA polymerase β's purified from mouse myeloma MOPC104E, chick embryo, and cherry salmon testis. The major polypeptide bands of these enzymes were detected at positions corresponding to molecular weights between 38K daltons and 40K daltons. It should be noted that mouse DNA polymerase β seems to be slightly larger than the chick enzyme, while cherry salmon enzyme is slightly smaller than the chick one.

Lane e of Fig. 1, Panel A shows the result obtained for DNA polymerase β preparation partially purified from bullfrog ovary. Since this preparation was not purified extensively, it was impossible to identify the polypeptide of DNA polymerase β by the dye staining method. However, the frog enzyme was detected by an immunological method using anti-chick DNA polymerase β antibody. Panel B of Fig. 1 shows the immunoperoxidase staining of the nitrocellulose membrane to which partially purified bullfrog and chick DNA polymerase β's had been transferred electrophoretically from an SDS-polyacrylamide gel. The polypeptide of bullfrog enzyme was stained with anti-chick DNA polymerase β antibody at exactly the same position as that of chick enzyme.

The results obtained indicate that the sizes of polypeptides of DNA polymerase β's from all animals investigated are very similar, *i.e.*, about 40K daltons.

Detection of Structural Homology by an Immunological Method—Panel B of Fig. 1 shows that anti-chick DNA polymerase β antibody cross-reacts strongly with bullfrog enzyme. This suggests the existence of extensive structural homology between the chick and bullfrog enzymes. Cross-reaction between anti-chick DNA polymerase β and cherry salmon enzyme was also observed (Fig. 1, Panel C), though the stained band was very thin. The results indicate that the structural homology between the chick and cherry salmon enzymes is lower than that between the chick and bullfrog enzymes. DNA polymerase β's from mouse myeloma and rat ascites hepatoma were also stained weakly by anti-chick β antibody (data not shown).

Figure 2 shows the results of neutralization of mouse and cherry salmon DNA polymerase β activities with anti-chick DNA polymerase β antibody. Both enzyme activities were neutralized partially. These results indicate the existence of partial structural homology among DNA polymerase β's of various vertebrate species.

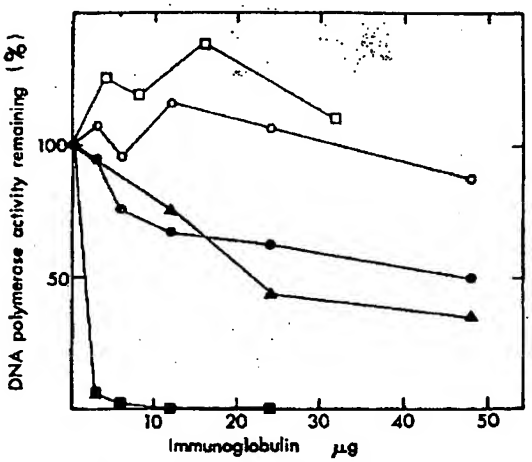
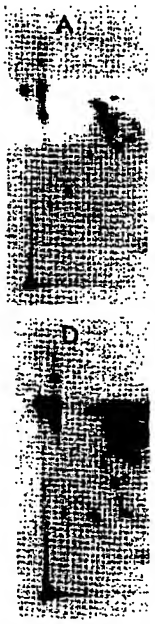


Fig. 2. Neutralization test of DNA polymerase β's from various animal sources by anti-chick DNA polymerase β antibody. Partially purified preparations of DNA polymerase β's from mouse myeloma MOPC104E, chick embryo, and cherry salmon testis were pre-incubated with the indicated amount of anti-chick DNA polymerase β antibody IgG or control serum γ-globulin at 0°C for 4 h, then the remaining enzyme activity was measured. ■, chick β+anti-chick β IgG; ●, mouse β+anti-chick β IgG; ▲, salmon β+anti-chick β IgG; □, chick β+control serum γ-globulin; ○, mouse β+control serum γ-globulin.

Detection of Structural Homology among Chick, Cherry Salmon, and Mouse DNA Polymerase β by Tryptic Peptide Mapping—The dye-stained polypeptide bands of about 40K daltons in SDS-polyacrylamide gels were cut out and the protein in each gel slice was radioiodinated with ^{125}I. The iodinated polypeptide was digested extensively with trypsin, and tryptic peptides were developed two-dimensionally on a silica gel-coated thin layer plate. Autoradiograms of the maps of chick (A), cherry salmon (B), and mouse (C) enzymes are shown in Fig. 3. Panel D is the peptide map of a mixture of chick and cherry salmon enzymes. Although many spots are specific to the chick enzyme and others are specific to the salmon enzyme, significant numbers of spots are common to both. At least 20 spots are common, while 12 are chick-specific and about 20 spots are



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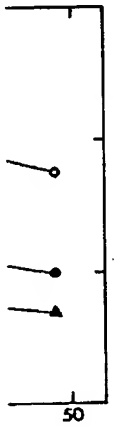

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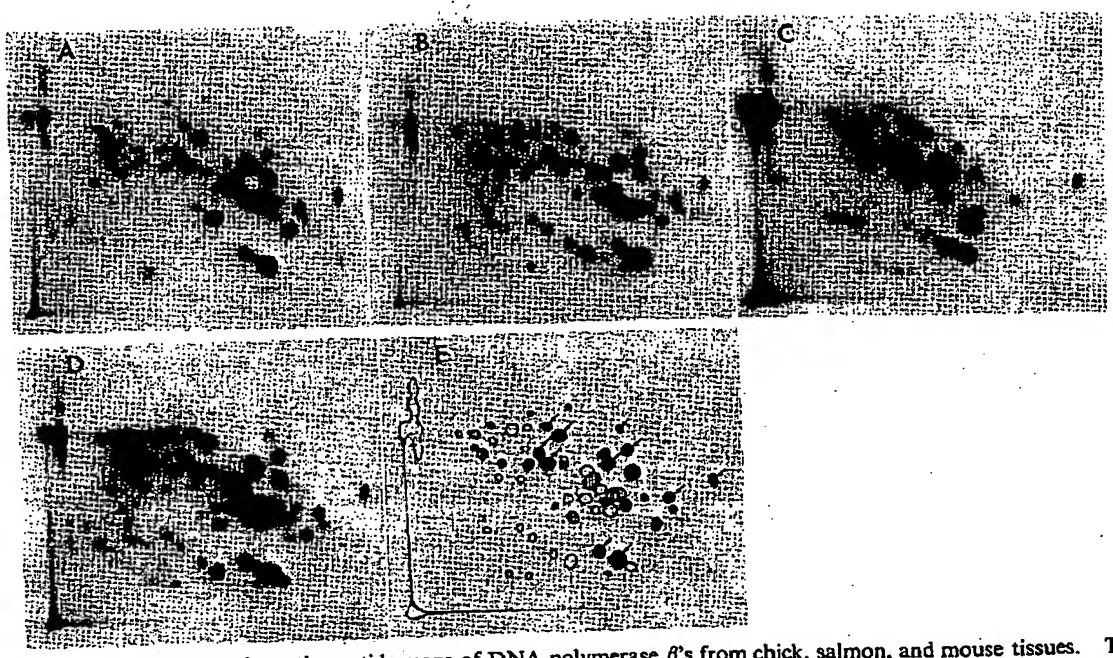

Fig. 3. Comparison of tryptic peptide maps of DNA polymerase β's from chick, salmon, and mouse tissues. The 40K daltons bands of DNA polymerase β's from chick embryo, cherry salmon testis, and mouse myeloma MOPC104E were cut from the electrophoresis gel shown in Fig. 1, Panel A. The enzyme protein in each gel slice was radioiodinated, and digested extensively with trypsin, then the tryptic peptides were developed two-dimensionally on a silica gel-coated thin layer plate, and autoradiograms were taken. Panel A: tryptic peptide map of chick β. Panel B: salmon β. Panel C: mouse β. Panel D: mixture of chick and salmon β (tryptic products of chick β and salmon β were mixed and developed). Panel E: traced diagram of Panel D. Solid spots are those common to the chick and salmon enzymes; striped spots are those present in the map of chick enzyme (Panel A) but not in that of salmon enzyme (Panel B); open circles show salmon-specific spots and dotted lines show minor spots (not scored). Solid spots indicated by arrows are also present in the map of mouse enzyme (Panel C).

salmon-specific, as shown in the traced figure (Panel E). Arrows in Panel E indicate the spots common to the mouse enzyme, a peptide map of which is shown in Panel C. It is noteworthy that the majority of the common spots of chick and salmon enzymes are also common to the mouse enzyme.

DISCUSSION

Our previous experiments on the structure of DNA polymerase β from mammalian cells (*3*) and chick embryo (*4*) demonstrated extensive structural homology not only among various mammalian enzymes but also between the rat and chick enzymes. The experiments reported here are an extension of the above studies.

The apparent molecular weights of DNA polymerase β's from mammalian tissue, chick, frog, and fish sources as estimated by SDS-polyacrylamide gel electrophoresis are almost identical, though very slight size differences were detected among the enzyme from different animal classes. The enzymatic properties of these enzymes are well known to be very similar, and immunological cross-reactivities of anti-chick DNA polymerase β with the mammalian and fish enzymes were detected by immuno-peroxidase staining and neutralization tests. Chang *et al.* (*2*) also reported the conservation of molecular size and protein structure of this enzyme from mammals to parasitic protozoans based on immunological detection studies. All of these results suggest strong structural conservation of this enzyme.

Using the tryptic peptide mapping method, we obtained direct evidence for the structural

homology of this enzyme from various vertebrates. Our results reported here clearly demonstrate the existence of extensive common amino acid sequences in the primary structures of mouse, chick, and cherry salmon DNA polymerase β molecules. The common spots on peptide maps of these enzymes are the spots of tryptic peptides digested from the "constant region." This "constant region," the sequence of which is strongly conserved in evolution, is probably the essential region for the DNA polymerase β function.

In general, the molecular structure of an enzyme or a protein factor essential for the survival of an animal cell is thought to be conserved throughout biological evolution. DNA polymerase β is believed to be responsible for the repair of damaged DNA (*12*, *13*), and this must be an essential cellular function. If the function of this enzyme cannot be replaced by any other enzyme, then the functionally important region in the structure of DNA polymerase β would be expected to be strongly conserved. The "constant region" shown by the peptide mapping method is presumably the essential region for the function of the DNA polymerase β molecule. The amino acid sequence of this region has been strongly conserved for more than three hundred million years of evolution from fish to mammals. On the other hand, the structure of the non-essential part of the enzyme molecule has changed to some extent as judged from the species-specific spots in the peptide maps.

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